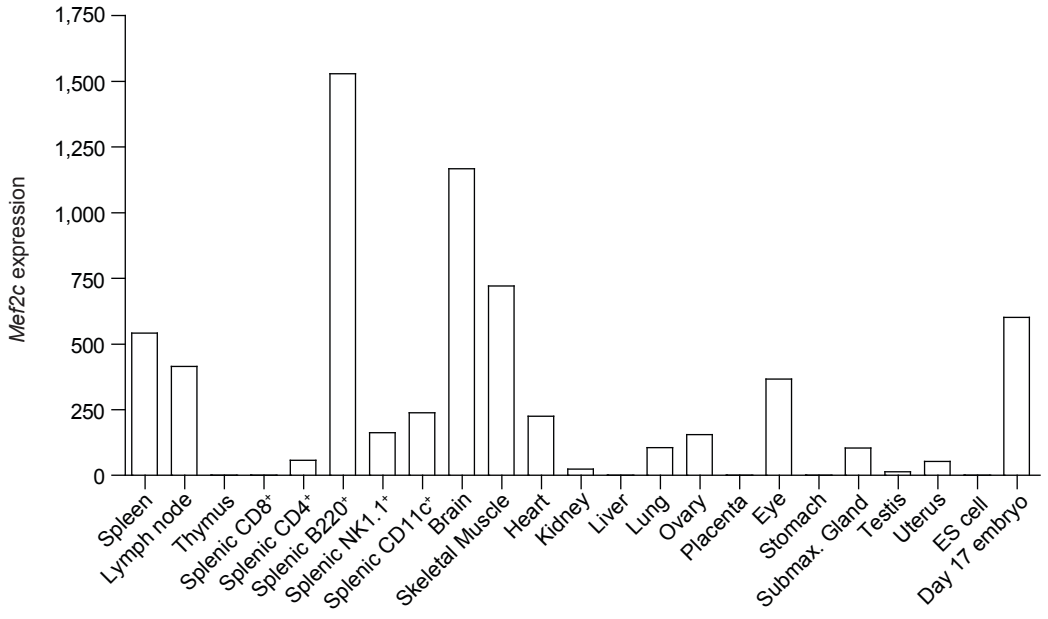
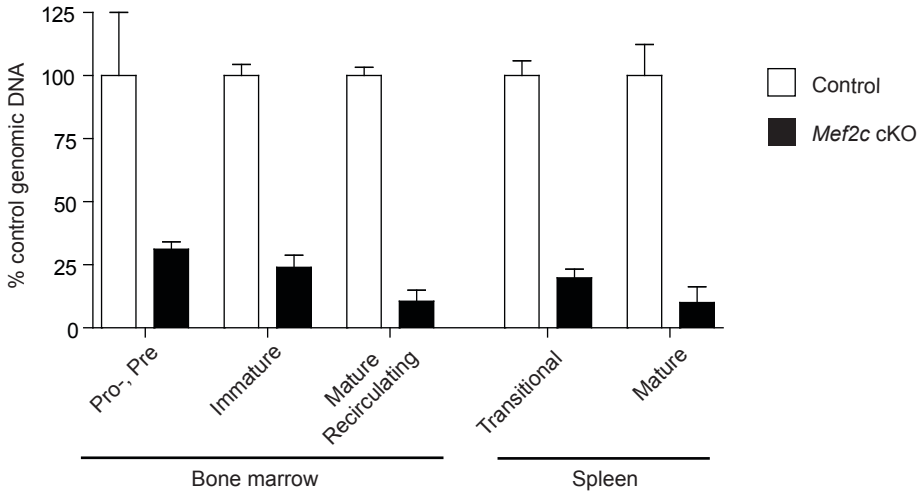
Supplementary Figure 1



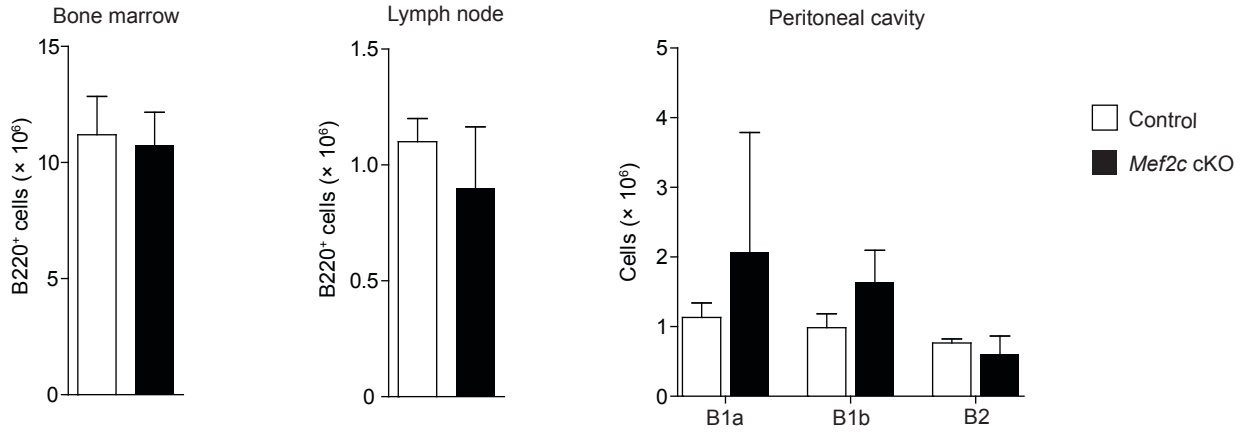
Supplementary Figure 1 *Mef2c* is highly expressed in B cells compared to a variety of murine tissues. RNA from the indicated murine tissues was harvested and used for gene expression microarray profiling using Affymetrix GeneChip Mouse Genome 430 2.0 arrays. Normalized and modeled expression values for *Mef2c* were generated using DNA-Chip analyzer (dChip) software and are expressed in arbitrary units.

Supplementary Figure 2



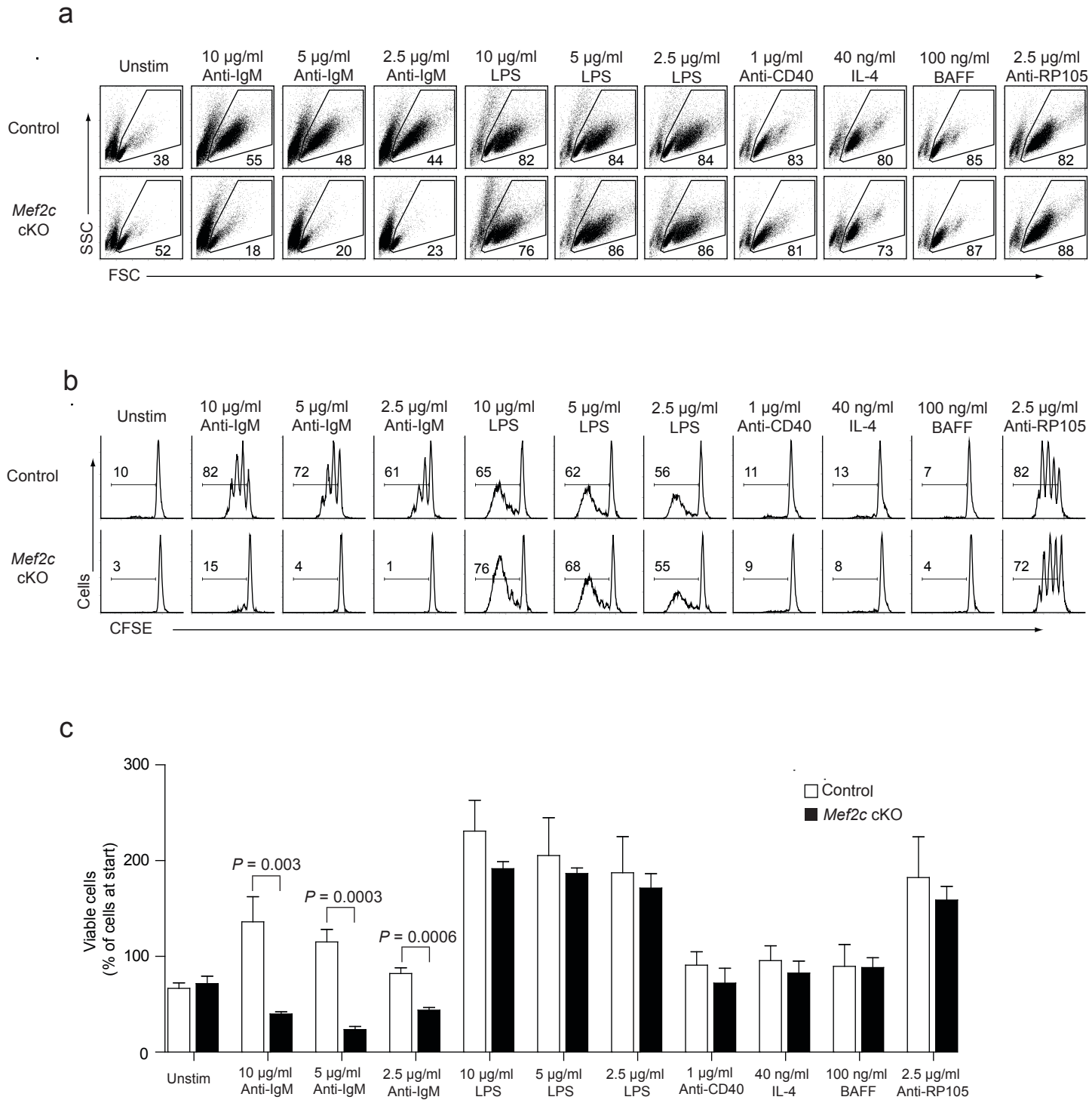
Supplementary Figure 2 Deletion of the *Mef2c*^{fl} allele in B cell subsets from the bone marrow and spleen. Quantitative real-time PCR of genomic DNA isolated from sort-purified B cell subsets was performed using primers that amplify a span of the *Mef2c* genomic sequence flanked by *lox*P sites that is excised by the CD19-driven Cre recombinase. Data are expressed as a percentage of the *lox*P-flanked genomic segment remaining in the indicated *Mef2c* cKO tissue compared to the corresponding control tissue. In the bone marrow, Pro-, Pre- B cells were defined as B220^{int} IgM^{-}, Immature B cells were defined as B220^{int} IgM^{hi}, and Mature B cells were defined as B220^{hi} IgM^{int}. In the spleen, Transitional B cells were defined as B220^{+} AA4.1^{+} and Mature B cells were defined as B220^{+} AA4.1^{-}. Data are the mean ± S.D. of triplicate samples from an experiment in which bone marrow cells or splenocytes from 3 mice per group were combined to sort-purify the indicated B cell subsets for genomic DNA analysis.

Supplementary Figure 3



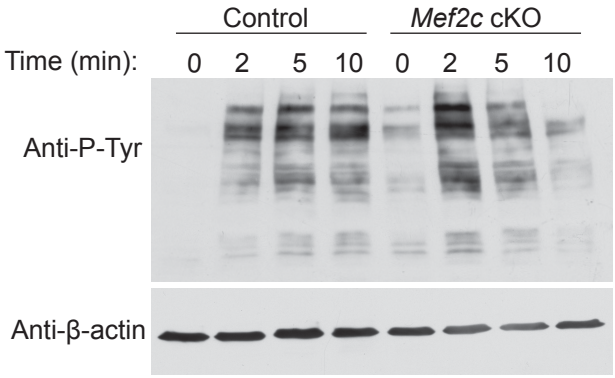
Supplementary Figure 3 Total cell numbers in bone marrow, inguinal lymph nodes, and peritoneal cavity in control and *Mef2c* cKO mice. Cells from the femurs, inguinal lymph nodes, and peritoneal cavity of control and *Mef2c* cKO were counted and stained with anti-B220 for bone marrow cells and inguinal lymph node cells, or anti-CD5, anti-B220, and anti-CD11b for peritoneal cells. Total numbers of $B220^+$ cells in the bone marrow and inguinal lymph nodes were determined based on the percentage of $B220^+$ cells and on total cell numbers in the respective tissue. Total numbers of B1a ($B220^+$ $CD11b^+$ $CD5^+$), B1b ($B220^+$ $CD11b^+$ $CD5^-$), and B2 ($B220^+$ $CD11b^-$) B cells were determined based on the frequency of each cell type and the total number of cells recovered from the peritoneal cavity. Data are the mean ± S.D. of at least three mice per group.

Supplementary Figure 4



Supplementary Figure 4 *Mef2c* is required for B cell survival and proliferation in response to BCR stimulation, but not for responses to other B cell stimuli. (a-b) B cells from control and *Mef2c* cKO spleens were labeled with CFSE and cultured without stimulation (Unstim) or with the indicated stimulus for 72 hours. (a) Viable cells were identified based on forward and side scatter characteristics. (b) The percentage of cells having undergone at least one cellular division was measured based on CFSE dye dilution. Data in (a) and (b) are representative of at least two independent experiments. (c) B cells from control and *Mef2c* cKO spleens were stimulated as in (a-b) for 72 hours and viable cells were counted and reported as a percentage of the number of cells present at the start of culture. Data shown in (c) is the mean ± S.D of three mice per group. Statistically significant differences (P 0.05) are indicated.

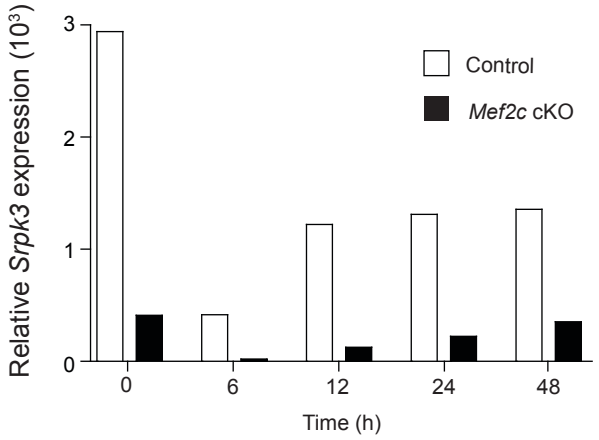
Supplementary Figure 5



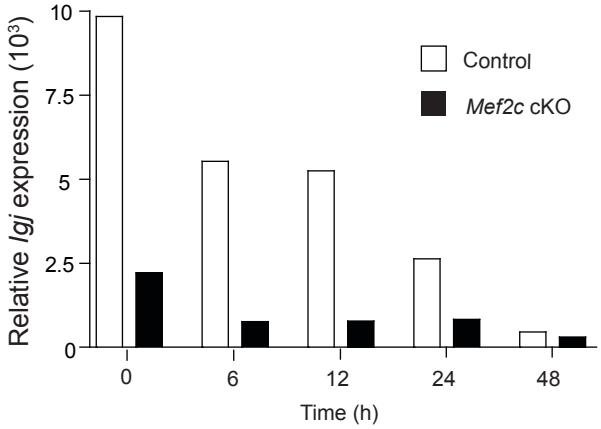
Supplementary Figure 5 Patterns of tyrosine phosphorylation in B cells following BCR stimulation. B cells from control and *Mef2c* cKO spleens were stimulated with anti-IgM (10μg/ml) for 0, 2, 5, or 10 minutes and stimulations were stopped by pelleting and lysing cells on ice for 1 hour in RIPA lysis buffer (150 mM NaCl, 1.0% NP-40, 0.5% deoxycholic acid, 0.1% sodium dodecyl sulfate, 50 mM Tris (pH 8.0)) containing 1 mM PMSF, 1 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM NaF_3, and 1 mM $NaVO_4$. Lysates were separated under reducing conditions by gel electrophoresis, transferred to a nitrocellulose membrane, and probed using a horseradish peroxidase-conjugated phosphotyrosine-specific antibody (clone RC20, BD Transduction Laboratories). β-actin levels are shown as a loading control.

Supplementary Figure 6

a

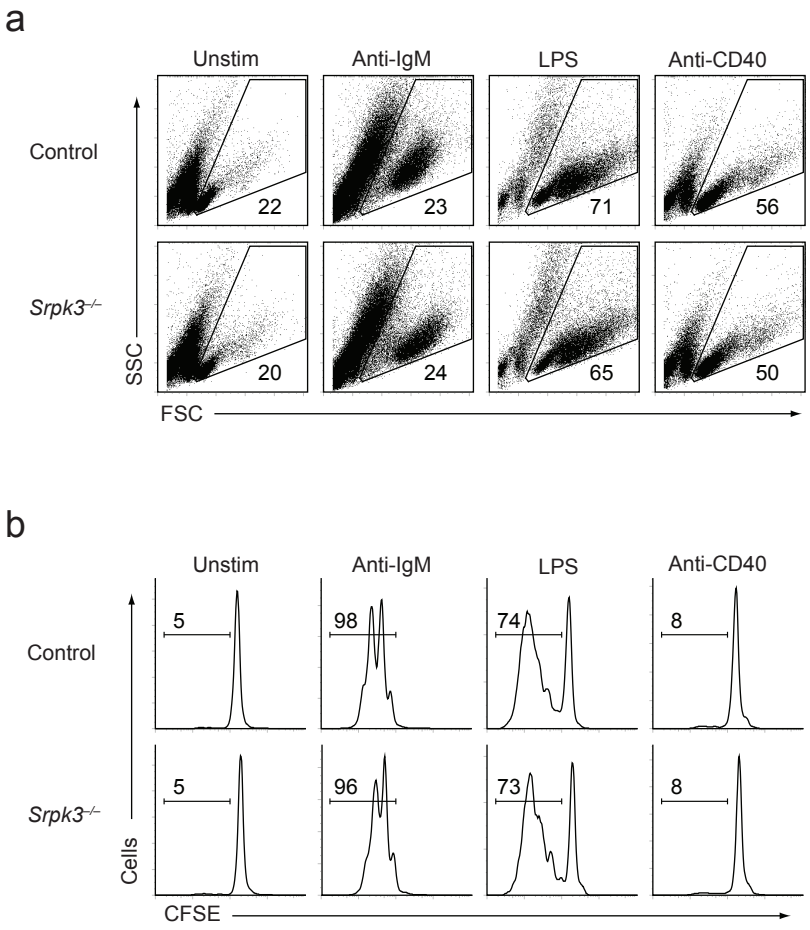


b



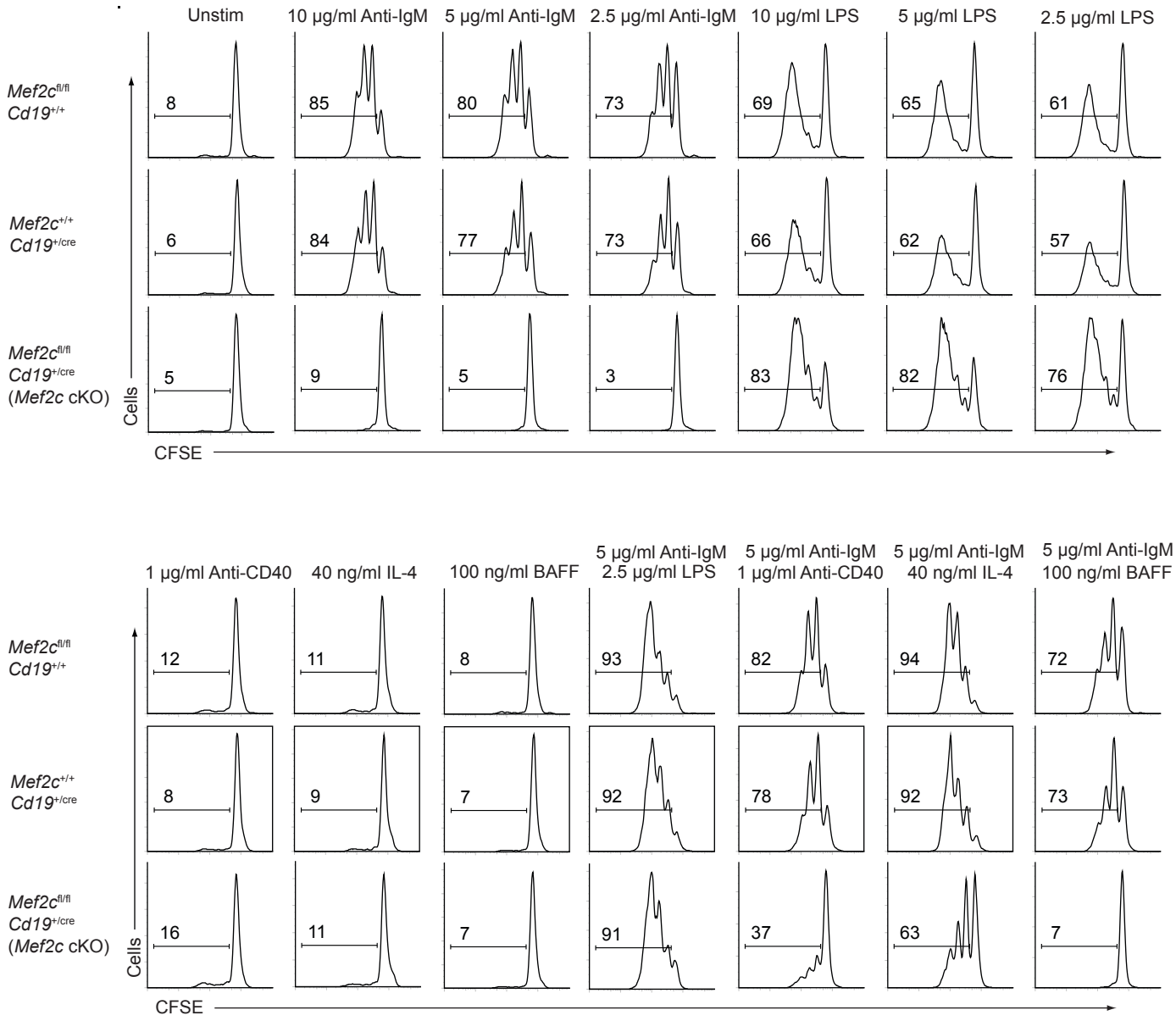
Supplementary Figure 6 Expression of serine/arginine-rich protein specific kinase 3 (*Srpk3*) and immunoglobulin joining chain (*Igj*) is dependent on *Mef2c*. B cells from control and *Mef2c* cKO spleens were cultured for 0, 6, 12, 24, or 48 hours with anti-IgM (5 μg/ml) and RNA was harvested for gene expression microarray analysis using Affymetrix GeneChip Mouse Genome 430 2.0 arrays. Normalized and modeled expression values for *Srpk3* (a) and *Igj* (b) were generated using DNA-Chip analyzer (dChip) software and are expressed in arbitrary units.

Supplementary Figure 7



Supplementary Figure 7 *Srpk3* is not required for B cell survival and proliferation in response to BCR stimulation. B cells from control and *Srpk3*$^{-/-}$ spleens were labeled with CFSE and cultured without stimulation (Unstim), with anti-IgM (5 μg/ml), LPS (5 μg/ml), or anti-CD40 (1 μg/ml) for 72 hours. (a) Viable cells were identified based on forward and side scatter characteristics. (b) The percentage of cells having undergone at least one cellular division was measured based on CFSE dye dilution.

Supplementary Figure 8



Supplementary Figure 8. *Mef2c*$^{fl/fl}$ *Cd19*$^{+/+}$ and *Mef2c*$^{+/+}$ *Cd19*$^{+/cre}$ B cells proliferate comparably to various B cell mitogens, whereas *Mef2c*$^{fl/fl}$ *Cd19*$^{+/cre}$ (*Mef2c* cKO) B cells display a severe proliferative defect to anti-IgM stimulation. B cells from *Mef2c*$^{fl/fl}$ *Cd19*$^{+/+}$, *Mef2c*$^{+/+}$ *Cd19*$^{+/cre}$, and *Mef2c*$^{fl/fl}$ *Cd19*$^{+/cre}$ (*Mef2c* cKO) spleens were labeled with CFSE and cultured without stimulation (Unstim) or with the indicated stimulus or stimuli for 72 hours. The percentage of cells having undergone at least one cellular division was measured based on CFSE dye dilution.